

AMENDMENTS TO THE SPECIFICATION

With the paragraph starting on page 4, line 20, please amend the specification as follows:

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Capillary scale systems also are shown in United States patent No. 6,194,900, the entire disclosure of which is incorporated herein by reference for all purposes. In such systems, a capillary-based analyte extraction chamber is connected to an NMR flow site, such as by being positioned as an operation site along a capillary channel extending to the NMR flow cell.

Small volume flow probes are shown, for example, by Haner et al. in *Small Volume Flow Probe for Automated Direct-Injection NMR Analysis: Design and Performance*, J. Magn. Reson., 143, 69-78 (2000), the entire disclosures of which is incorporated herein by reference for all purposes. Specifically, Haner et al show a tubeless NMR probe employing an enlarged sample chamber or flowcell. Microcoil-based micro-NMR spectroscopy is disclosed in United States patent No. 5,654,636, United States patent No. 5,684,401, and United States patent No. 6,097,188, the entire disclosures of all of which are incorporated herein by reference for all purposes. Sample amounts can now range as small as several hundred microliters for conventional flowprobes to smaller than 1 uL for microcoil-based capillary-scale flowprobes. Acquisition times typically range from minutes to hours. The most expensive and technologically limiting component of the NMR system is the superconducting magnet. Although significant financial and technical investment has been made in the development of elaborate mechanical (robotic-controlled) sample changers and, more recently, automated flow injection systems for repetitive and continuous sample throughput, the magnet remains today a dedicated component in which only sequential, one-at-a-time analysis of samples is carried out.

With the paragraph starting on page 8, line 22, please amend the specification as follows:

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The multiple NMR sites optionally can be optimized for different nuclear species and/or for 1 or 2 dimensional NMR study, e.g., the sites can be optimized similarly or differently, using different materials, such as fused silica and PEEK, fused silica and ~~polytetrafluoroethylene~~ polytetrafluoroethylene and/or other suitable materials known to those skilled in those skilled in the art.

With the paragraph starting on page 9, line 20, please amend the specification as follows:

\_\_\_\_ Fig. 1 is a schematic block diagram of a preferred embodiment of the NMR system disclosed here;

\_\_\_\_ Fig. 2 is a schematic illustration of a preferred embodiment of a NMR probe and other components of the system of Fig. 1;

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\_\_\_\_ Fig. 3 is a schematic view, partially broken away, of a NMR detection site of the system of Fig. 1 in a preferred orientation relative to the module; module

\_\_\_\_ Fig. 4 is an enlarged schematic view, partially broken away, of the sample holding void and associated microcoil of the NMR detection site of Fig. 3, showing a further enlarged view of same;

\_\_\_\_ Fig. 5 is a perspective view of the enlarged void of a NMR detection cell in accordance with a preferred embodiment, the NMR microcoil being broken away;

\_\_\_\_ Figs. 6-8 are schematic illustrations of alternative preferred embodiments of NMR probe modules, each showing detection site orientation, with the top of the page being upward into an NMR magnet;

\_\_\_\_ Fig. 9 is a schematic perspective view of an embodiment of a NMR probe, showing the orientation of a probe module within the probe;

\_\_\_\_ Fig. 10 is a schematic perspective view of an embodiment of a NMR probe, showing in enlarged break-out view the orientation of a probe module within the probe; module

\_\_\_\_ Fig. 11 is a schematic perspective view of an embodiment of a NMR probe comprising operative components in communication with a probe module, showing in enlarged break-out view the orientation of the probe module within the probe;

\_\_\_\_ Fig. 12 is a schematic cross-sectional elevation view of a fluid-handling substrate suitable for use as a NMR detection module as disclosed here;

\_\_\_\_ Fig. 13 is a schematic cross-sectional view of four alternative configurations for fluid channels in a probe module;

\_\_\_\_ Fig. 14 is a schematic view of an NMR detection site in a probe module; and

\_\_\_\_ Fig. 15 is a schematic view of a probe module, showing preferred placement and orientation of impedance matching elements.

**With the paragraph starting on page 13, line 19, please amend the specification as follows:**

Referring now to the drawings, Figure 1 is a schematic representative of an electrofluidic system in accordance with the present disclosure. A multiplicity of sample management modules are in operative electrical and fluidic communication with a multiplicity of primary stage detectors, a peak management module, and a multiplicity of ancillary stage detectors. Sample introduction can be from a variety of introduction means well known to those skilled in the art, and may include autosamplers with or without additional means of solid phase extraction. In general, the flow of information and fluid transport depicted in Figure 1 can proceed in either direction. For example, with the appropriate plumbing as understood by

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those skilled in the art, a storage loop used for sample introduction can be reused for sample storage, e.g. as a fraction collector at the end of the experiment. Furthermore, the figure should be considered sufficiently general as to represent the combination of any number of individual components, for example, the case where a single sample management module is used with a multiplicity of ancillary stage detectors. The sample management platforms are sufficiently sophisticated to be in operative electrical and fluidic communication with each other. One embodiment of this configuration is where each of the detection stages are NMR microcoil detectors. A preferred embodiment is where all detectors are integrated into the probe manifold but are not limited to NMR, e.g. UV, IR, and other NMR-compatible (predominantly non-magnetic) means of detection. The peak management module can include sample storage and routing capabilities, but can also include a means of sample management, e.g. solid phase extraction. In a most preferred embodiment, the components shown are predominantly integrated into a probe module, such as those of Figs. 3-8, with intelligent control of the overall processes being directed at least in part by electrical and fluidic processing elements in (i.e., on-board) the probe, e.g. microprocessors in operative communication with the detectors and fluidic management components shown in the drawings.

Fig. 2 illustrates a preferred embodiment of the system of Fig. 1, including a means of sample management (Waters CapLC and Sparc autosampler) external to the NMR probe, a primary stage detector (Waters photodiode array) external to the NMR probe, and a capillary-based NMR detection probe in operative electrical and fluidic communication with the sample management system and with the NMR spectrometer for effective control of analysis through a computer external to the probe. In an alternative preferred embodiment, some or all of these components would reside in (i.e., on-board) the probe, or even be provided as micro-scale components integrated on-board a substrate-based module, thereby improving

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efficiency, electrical and fluidic integrity, and promoting integration and complexity.

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With the paragraph starting on page 20, please amend the specification as follows:

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The multiple NMR sites are provided to allow for increased functionality and/or throughput. With multiple NMR sites the user is able to perform multiple NMR tests simultaneously which ~~increased~~increases the rate in which results may be obtained. Furthermore the NMR detection sites may be optimized for different types of testing allowing a single probe to be used for a number of tests. In some embodiments each NMR site maybe in fluid communication with multiple channels of the fluid pathway. In accordance with preferred embodiments, the NMR detection sites further comprise matching capacitors and tuning capacitors, fluid connectors and data transmission means such as signal carrying leads or the like. An example of an NMR detection site can be seen in Fig. 14. Other embodiments will be apparent to one skilled in the art given the benefit of this disclosure.

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With the paragraph starting on page 22, line 10, please amend the specification as follows:

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The functionality of the NMR probe module is dependent, in part, upon the reception of separate signals from the individual coils (and impedance matching networks) that comprise the NMR detection site. Regardless of the form of signal acquisition (independent acquisition using multiple receivers, or time-multiplexed acquisition using RF switches), quasi-simultaneous acquisition demands a high degree of isolation between the microcoils and matching circuits at each nuclear frequency of interest.

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The coils can be positioned with spatial separation and orthogonal geometric orientation to reduce coupling. Similarly, the impedance matching circuits can be shielded and positioned in the probe in such a manner to reduce coupling.—

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With the paragraph starting on page 24, line 4, please amend the specification as follows:

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Li et al. (Li 1999 Anal. Chem. 71 4815-4820) describes a 4-coil assembly illustrative of certain aspects of the present disclosure. The solenoidal microcoils (diameter = 360 ~~um~~, length approx. 1 mm) are mounted on horizontal (transverse to  $B_0$ ) capillaries with a 90 degree rotation (x, y) and 5 mm vertical spacing between adjacent coils. Additional details of basic construction are known generally, as shown in the Li et al. reference mentioned above ~~and incorporated herein by reference for all purposes.~~ In the example described in the Li et al. reference, a four-coil system was constructed for operation in a 250 MHz wide-bore (89 mm) magnet; a two coil system could be accommodated in either a narrow bore or a wide bore 500-MHz magnet. For the four coil system, each coil was fabricated identically using 17 turns of 50 um-diameter copper wire with a 6 um-thick polyurethane coating (California Fine Wire Co., Grover Beach, CA) wrapped around 355-um outer diameter (o.d.), 180 um-inner diameter (i.d.) polyimide-coated fused-silica capillary (Polymicro Technologies, Phoenix, AZ), giving an observe volume ( $V_{obs}$ ) of 28 nL. Teflon flow tubes were attached to both ends of the capillary for sample loading. The coils were mounted on printed circuit boards, and impedance matching capacitors were added in a balanced configuration. For each individual circuit, the matching capacitors were a 2.2-pF fixed capacitor (700A series American Technical Ceramics Corp., Huntingdon Station, NY) and variable capacitor (Gigatrim, 0.6-4.5 pF, Johanson Mfg. Co., Boonton, NJ). The tuning capacitor was a single variable capacitor of the type described above. The microcoils were mounted on above the other with a

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vertical spacing of 5 mm between adjacent coils. Alternate coils were rotated 90° with respect to each other to reduce coupling. The matching networks were also placed at 90° to each other, again to reduce coupling. The whole system was surrounded by a container filled with FC-43. The outer diameter of the assembly was 4.5 cm. For the two-coil system at 500 MHz, the microcoils were constructed as described above, one on a 75  $\mu\text{m}$ -i.d., 360  $\mu\text{m}$ -o.d. capillary ( $V_{\text{obs}}$  5 nL) and the other on a 200- $\mu\text{m}$  i.d., 360  $\mu\text{m}$ -o.d. capillary ( $V_{\text{obs}}$  31 nL). The coils were then mounted on double-sided printed circuit boards. The capillaries were oriented at the magic angle with respect to the  $B_0$  field, and the two boards attached back-to-back with copper shielding between the boards. The matching networks were designed to maximize the distance between the elements of the two circuits, with the microcoils separated by 5 mm transversely with respect to the  $B_0$  field. Impedance matching, flow tube attachment, and susceptibility matching were performed as for the 250 MHz probe. The outer diameter of the assembly was 2.9 cm, allowing its use in a standard narrow-bore magnet. In the Li et al. example, the probe was used to carry out lower-field experiment on a 250 MHz wide-bore (89 mm) magnet (Oxford, Instruments, Oxford, England) using a Macintosh controlled Libra console (Tecmag, Houston, TX) running MacNMR 5.6. Higher-field NMR spectroscopy was performed on a Unity Inova spectrometer (Varian NMR Instruments Palo Alto, CA) with a 500 MHz widebore (89 mm) magnet (Oxford Instruments, Oxford, England). Data was processed with the NUTS software package (Acorn NMR, Inc., Fremont, CA). The NMR probe modules disclosed here differ, for example, from such earlier devices in having multiple detectors, each having a detection site, i.e., as described above, void in the capillary microchannel to receive a test sample, and having an NMR microchannel aligned therewith.

With the paragraph starting on page 26, line 1, please amend the specification as follows:

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The microchannels and associated NMR microcoils can be formed in a module, preferably a multi-layer substrate, such as a laminated multi-layer substrate, e.g., a selectively welded multi-layer substrate as disclosed in copending United States patent application Serial No. 60/239,010 filed on October 6, 2000, the entire disclosure of which is incorporated herein by reference for all purposes. PCT/US01/31333 filed on October 5, 2001 and published as WO0228532 claims priority to U.S. Application Serial No. 60/239,010. U.S. Application No. 10/033,315 (Pub. No. 2002/0176804), is a continuation of the PCT Application. Microlithographic microcoils can be employed in such laminate substrates, such as those disclosed in the above-mentioned US patent 5,684,401, the entire disclosure of which is incorporated herein by reference for all purposes. Alternatively, or in addition, one or more of the multiple NMR detector sites formed in the probe can be formed in a finger or peninsula-type extension of the substrate, and the microcoil can be formed as a separate 3-dimensional structure fitted over such substrate projection. It will be within the ability of those skilled in the art, that is, those skilled in this area of technology, given the benefit of this disclosure, to employ alternative suitable fabrication techniques for production of the multi-microcoil NMR detection probes disclosed here.

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